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## FIGURES

-Fig. 1.

A

TatA (Eco)	M- <del>GGISI</del> WQELITIAVIVVHLGFKKKLG-----	26
TatE (Eco)	M-GEISITKLLVVAALWVILFGTKKLR-----	26
TatAy (Bsu)	<del>---</del> PIGPCSLAVIAITVAVIPICPKKLP-----	25
TatAd (Bsu)	MFSNIGTPGCLLTFVIAIPICPSSKLP-----	27
TatAc (Bsu)	<del>M-</del> ELSFTKILVILFVGFLVCPDKLP-----	25
TatB (Eco)	<del>ME-</del> DIGESELLIIVELIICDYLICBQRLPVAVKTVAAGWIRALRSLATTVQNELTQELKLQ	49
	*	
TatA (Eco)	-----SIGSDLGASIKGFKKKAMSDE-----PKQDKTSQDADFTAKTI	64
TatE (Eco)	-----TLGGLGAAIKGFKKKAMNDDE-----A-AAKGADVDLQAELK	63
TatAy (Bsu)	-----ELGKAAGDTLREFKNATKGLT-----SDEEEKKKKEDQ-----	57
TatAd (Bsu)	-----EIGRAAKRTLLEFKSATKSLV-----SGDEKEEKSAELTAVK-	64
TatAc (Bsu)	-----ALGRAAGKALSEFKQATSGLT-----QDIRKNDSEN-----K-	57
TatB (Eco)	EFQDSLKKVEKASLTNLTPELKASMDELRQAAESMKRSYVANDPEKASDEAHTIHNP	114
	-----*	
TatA (Eco)	ADKOQADTNQE-----QAKTEDAKRHDKEQV	89
TatE (Eco)	SHKE-----	67
TatAy (Bsu)	-----	57
TatAd (Bsu)	-----QDKNAG	70
TatAc (Bsu)	-----EDKQM-	62
TatB (Eco)	VVKDNEAAHEGVTPAAAQTQASSPEQKPETTPEPVVKPAADAEPKTAAPSPSSSDKP	171

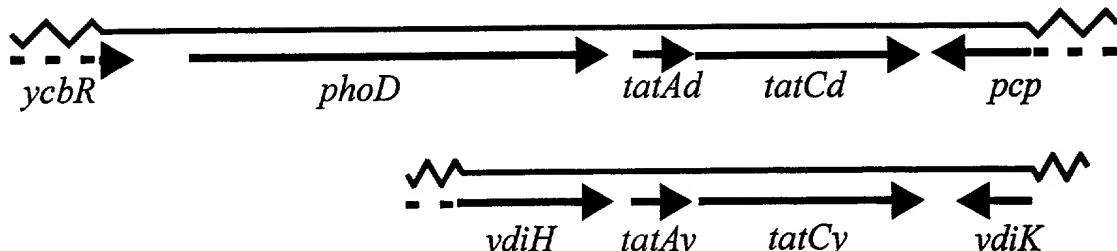
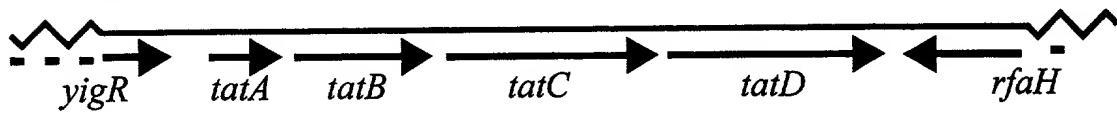
B

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Fig. 2.

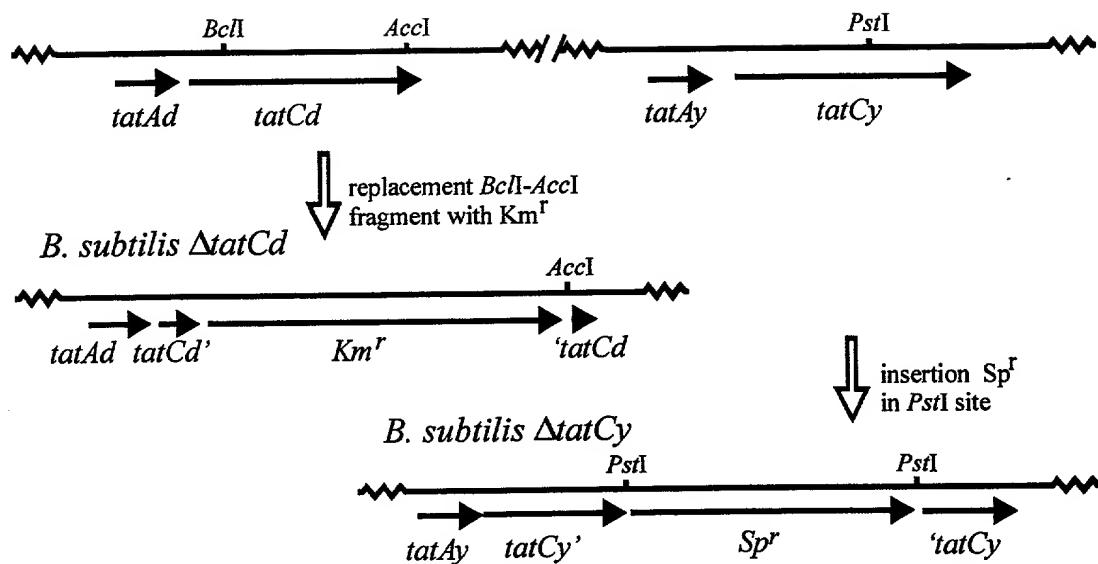
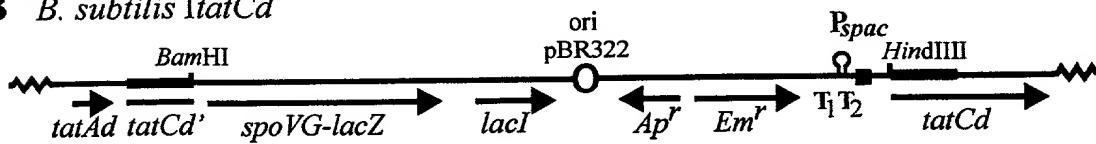
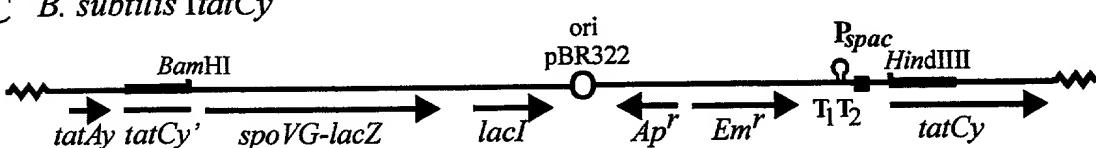
**A *B. subtilis*****B *E. coli***

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Fig. 3.

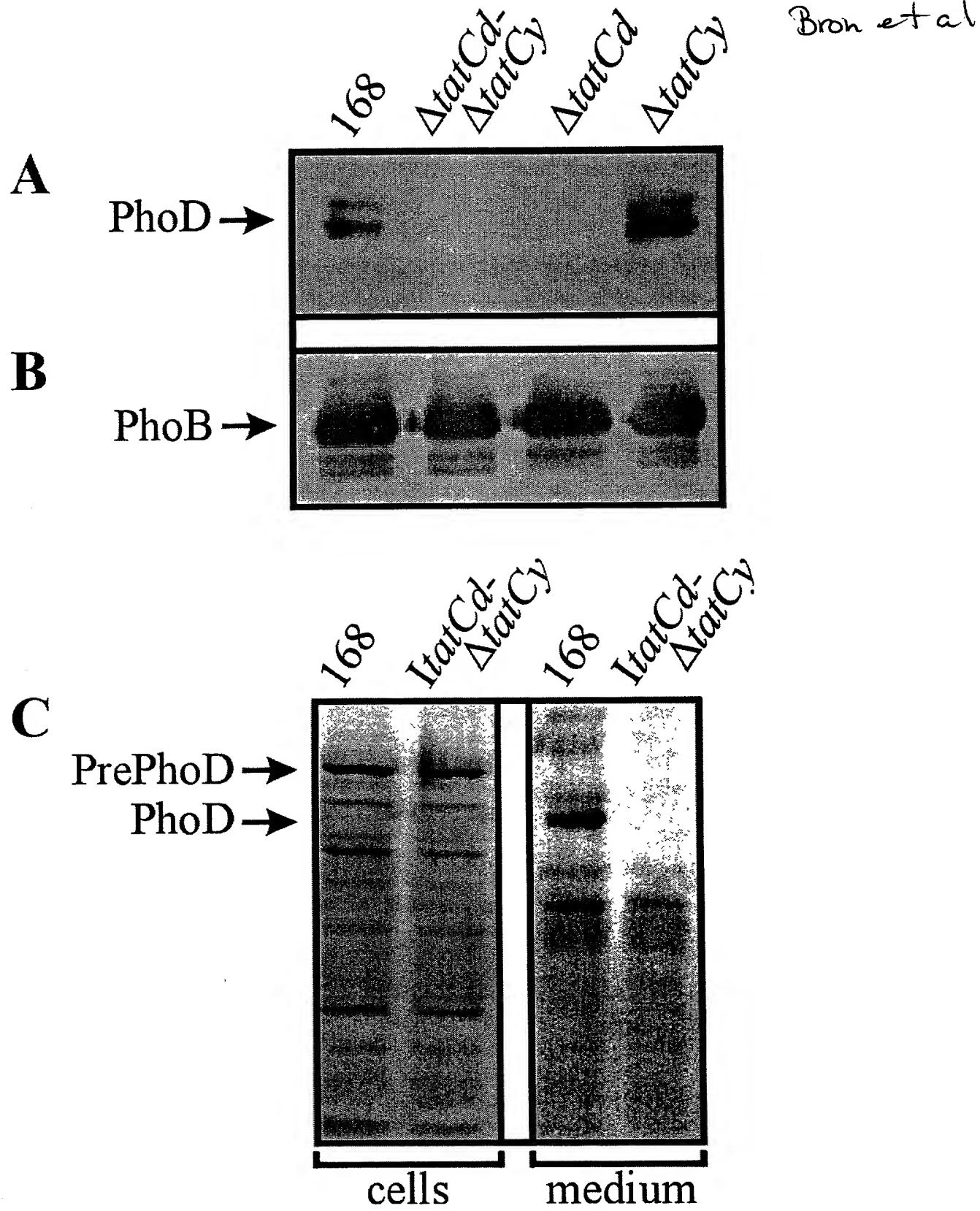
**A** *B. subtilis* 168**B** *B. subtilis*  $\Delta$ *tatCd***C** *B. subtilis*  $\Delta$ *tatCy*

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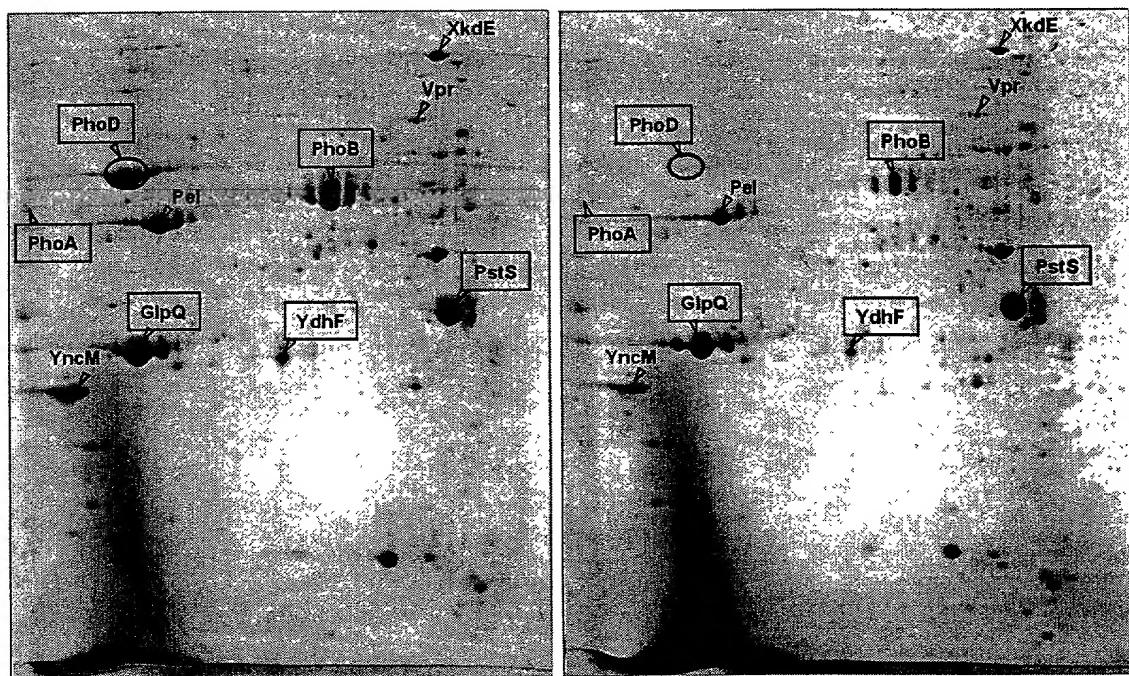
Fig. 4.



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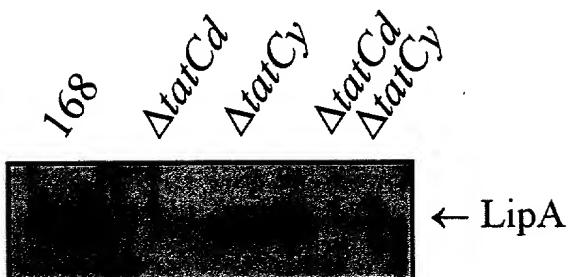
Fig. 5.

168

 $\Delta tatCd-\Delta tatCy$ 

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**FIGURE 6**



Tat-dependent secretion of the *B. subtilis* lipase LipA. *B. subtilis* 168 (parental strain), *B. subtilis*  $\Delta$ tatCd, *B. subtilis*  $\Delta$ tatCy, or *B. subtilis*  $\Delta$ tatCd- $\Delta$ tatCy were grown in TY-medium to end-exponential growth phase. To study the secretion of LipA, *B. subtilis* cells were separated from the growth medium by centrifugation. Proteins in the growth medium were concentrated 20-fold upon precipitation with trichloroacetic acid, and samples for polyacrylamide gel electrophoresis (SDS-PAGE) were prepared. Secreted LipA in the growth medium was visualized by SDS-PAGE and Western blotting, using LipA-specific antibodies.

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### FIGURE 7

Predicted twin-arginine (RR-)signal peptides of *B. subtilis*<sup>1</sup>

Protein	N	h	RR-Motif	H	h	C
AlbB	1	0.1	<b>RRILL</b>	27	2.0	AIA
AmyX <sup>TM</sup>	9	-0.8	<b>RRSFE</b>	15	1.1	-
AppB <sup>TM</sup>	8	0.5	<b>RRTLM</b>	19	2.3	-
LipA	7	-1.1	<b>RRIIA</b>	19	1.2	AKA
OppB <sup>TM</sup>	8	-0.6	<b>RRLVY</b>	24	2.0	-
PbpX	2	-2.2	<b>RRRKL</b>	14	2.9	WNA
PhoD	3	-1.3	<b>RRKFI</b>	17	0.9	VGA
QcrA <sup>TM</sup>	1	-1.1	<b>RRQFL</b>	19	1.3	-
TlpA <sup>TM</sup>	1	-0.8	<b>RRLII</b>	21	2.4	-
WapA <sup>W</sup>	1	-3.0	<b>RRNFK</b>	18	2.3	VLA
WprA	8	-1.7	<b>RRKFS</b>	20	1.9	AAA
YceA <sup>TM</sup>	1	-0.4	<b>RRAFL</b>	21	2.2	-
YesM <sup>TM</sup>	1	-1.5	<b>RRMKI</b>	20	2.4	QYA
YesW	1	-1.3	<b>RRSCL</b>	19	2.0	VKA
YfkN <sup>TM</sup>	1	-1.2	<b>RRTHV</b>	17	1.7	IHA
YkpC	8	-1.0	<b>RRVAI</b>	17	2.3	SLA
YkuE	1	-1.3	<b>RRQFL</b>	17	1.0	GYA
YmaC	7	0.0	<b>RRFLL</b>	15	2.4	YSL
YubF <sup>TM</sup>	9	-2.7	<b>RRNTV</b>	23	2.0	-
YuiC	8	0.2	<b>RRLLM</b>	20	1.9	IEA
YvhJ <sup>TM</sup>	2	-1.7	<b>RRKIL</b>	18	2.5	-
YwbN	1	-1.8	<b>RRDIL</b>	23	1.4	QTA

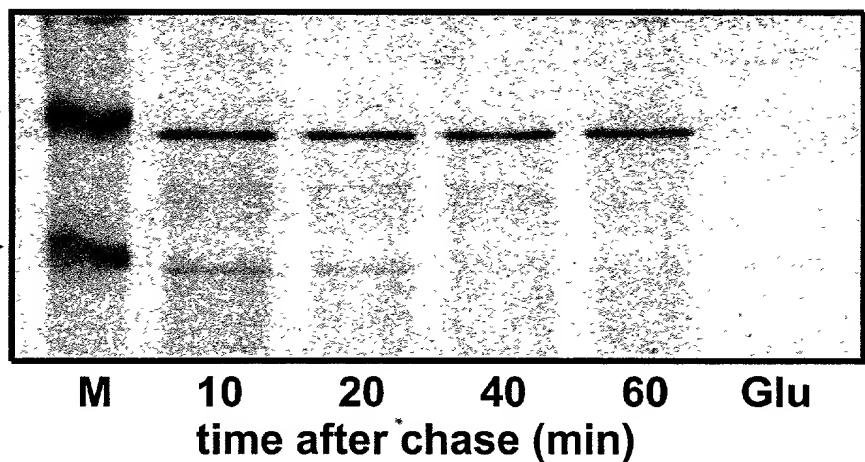
<sup>1</sup> The listed signal peptides contain, in addition to the twin-arginines, at least one other residue of the consensus sequence (R-R-X-Φ-Φ; printed in bold). The number of residues in the N- and H-domains of each signal peptide, and the average hydrophobicity (h) of each of these domains, as determined by the algorithms of Kyte and Doolittle (Kyte, J., and R. F. Doolittle [1982] A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.* 157:105-32), are indicated. Furthermore, the RR-motifs in the N-domain, and SPase I recognition sites in the C-domain (*ie.* positions -3 to -1 relative to the predicted SPase cleavage site) are shown. Proteins lacking a (putative) SPase I cleavage site, some of which contain additional transmembrane domains, are indicated with "TM". One protein containing cell wall binding repeats is indicated with "W".

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A

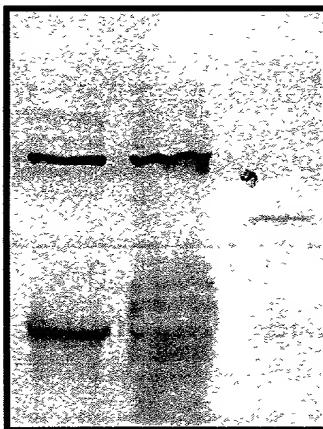
66 kDa →

46 kDa →



B

prePhoD →



SecB →

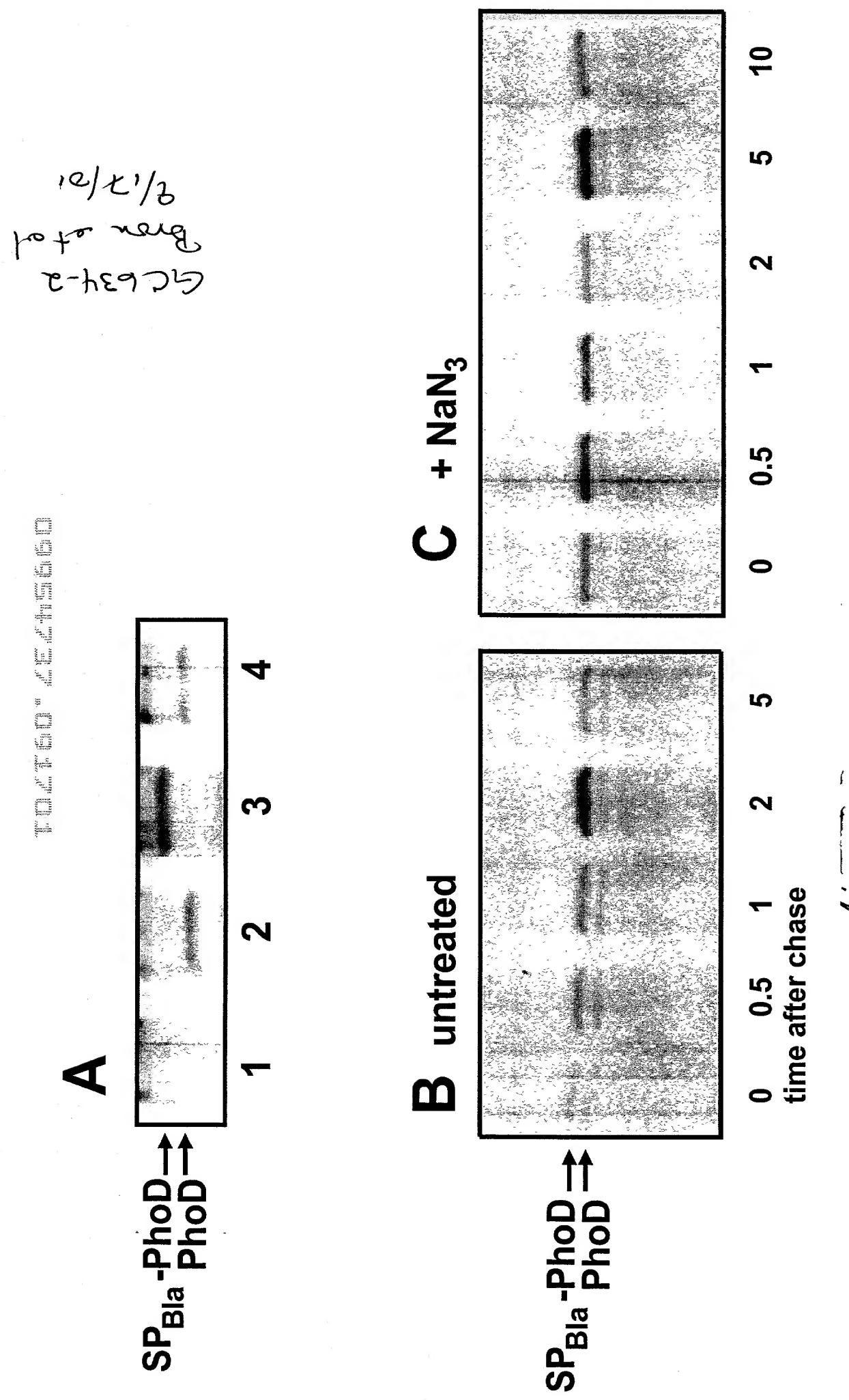


Proteinase K - + +

Triton X-100 - - +

Figure 8

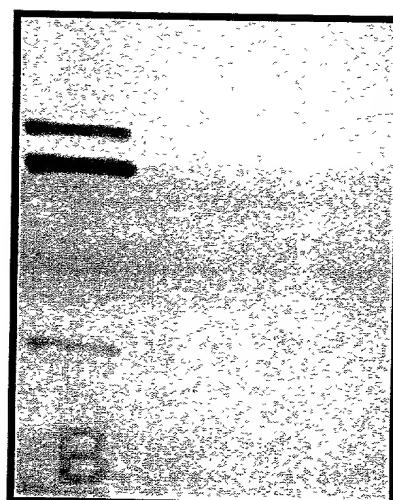
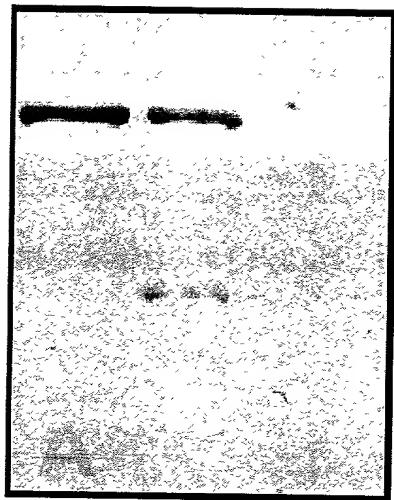
Figure 9



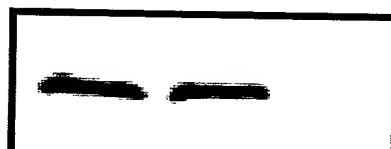
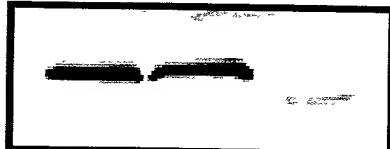
A

B

$\text{SP}_{\text{PhoD}}\text{-LacZ}$  →  
LacZ →



SecB →



Proteinase K  
Triton X-100

- + +  
- - +

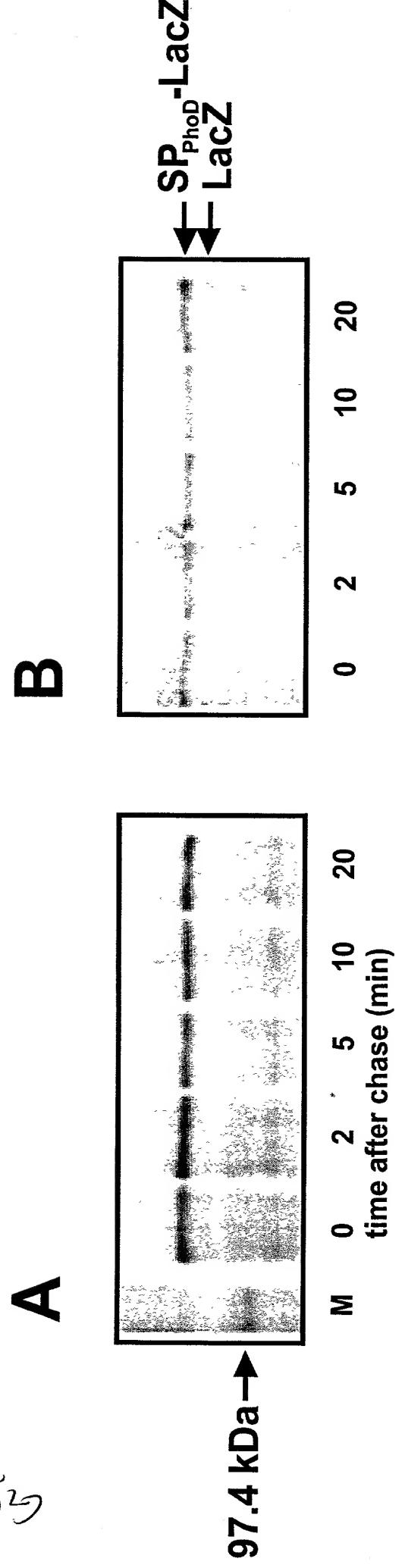
- + +  
- - +

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Figure 10

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Figure 11



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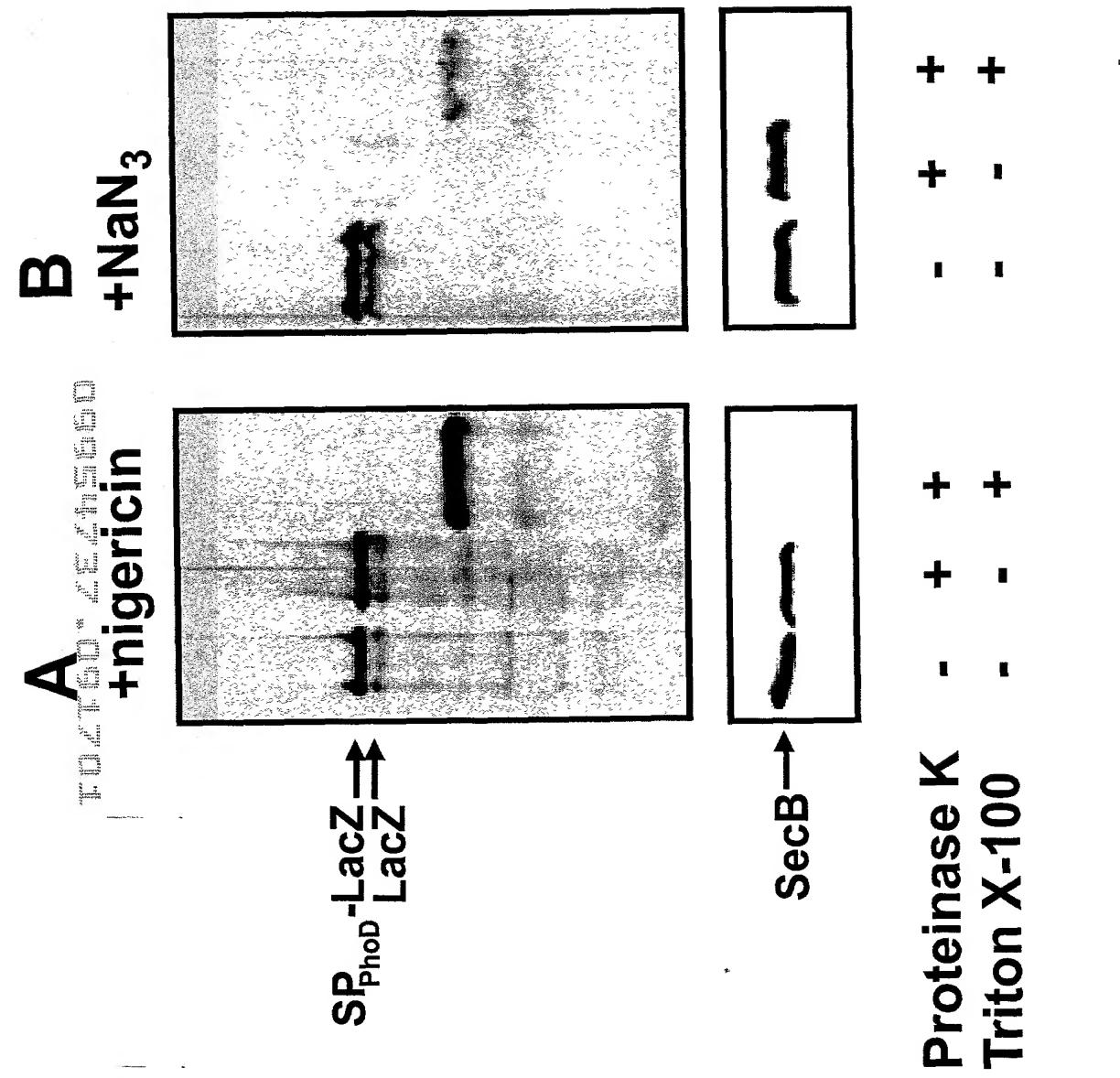
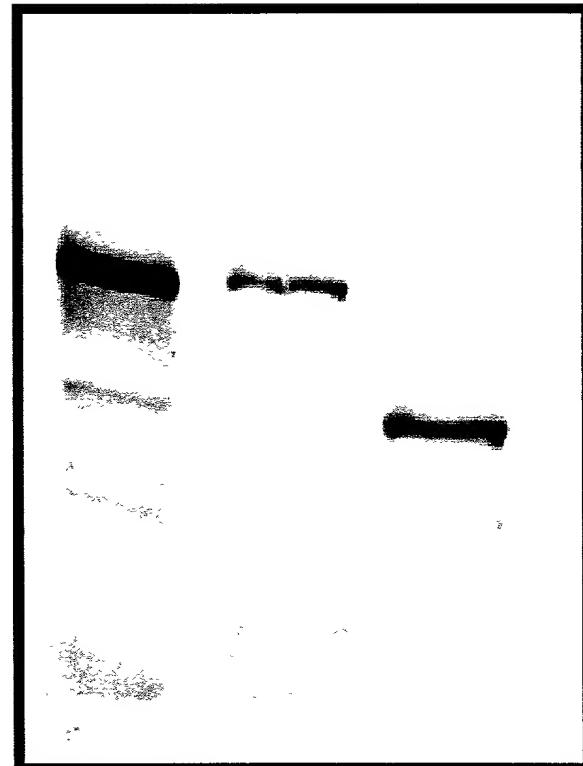


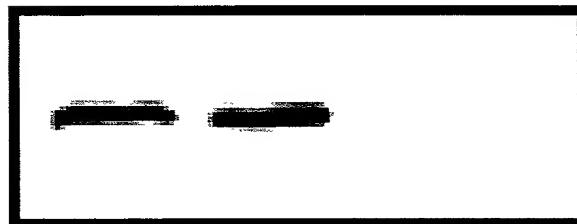
Figure 12

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**SP<sub>PhoD</sub>-LacZ →**



**SecB →**



**Proteinase K**

- + +

**Triton X-100**

- - +

**Figure 13**

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Figure 14  
Homologs in *B. alcalophilus*

TatA

**MGGLSVGSVVLIALVALLIFGPKKLPELGKAAGSTLREFKNATK  
GLADDDDDTKSTNVQKEKA**

TatC

**MTMMTPNQQTSKKKRKGRKGRVPMQDMSIMDHAEELRRRIF  
VVLAFFIVALIGGFFLAVPVITFLQNSPQAADMPFNAFRLTDPLRV  
YMNFAVITALVLIIPVILYQLWAFVSPGLKENEQKATLAYIPIAFL  
LFLAGIAFSYFILLPFVISFMGQMADRLEINEMYGINEYFSFLFQL  
TIPFGLLFQLPVVVMFLTRLGVVTPTFLRKIRKYAYFALLVIAGII  
TPPELTSHLFVTVPMLILYEISITISAITYRKYHGTTDHNGQESAK**